REMARKS

The Official Action dated November 27, 2001 and the references cited therein have been carefully reviewed. In view of the amendments presented herewith and the following remarks, Applicant respectfully requests favorable reconsideration and allowance of the application.

Brief Summary of the Prosecution

The present invention is directed to compositions and methods for anti-microbial biotherapy as alternatives to conventional pharmaceutical antibiotics. The invention provides novel antibacterial agents which are efficiently transferred to pathogenic bacteria, have a flexible range of potential hosts, and to which bacteria have difficulty developing resistance. The anti-microbial biotherapeutic compositions are preferably nonpathogenic bacterial donor cells harboring plasmids which are transferred conjugatively to pathogenic bacterial recipients wherein the plasmids exert a lethal effect.

In the Office Action dated November 27, 2001, the following rejections were made, or issues were raised.

1. Applicant was advised that the election of Group I claims was treated as an election without traverse. Applicant's election of claims 1-12, 14-17 and 29-30 was acknowledged, leaving those claims pending, while claims 13 and 28 were cancelled.

2. The Information Disclosure Statement filed August 30, 2000 was considered by the examiner.

3. Claims 1-12, 14-17 and 29-30 were rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention.

4. Claims 14 and 15 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for use of the term 'pre-determined route of administration'.

5. Claims 1-12 and 16-27 were rejected under 35 U.S.C. §102 (b) as allegedly anticipated by Metcalf et al. (Plasmid 35:1-13, 1996).

6. Claims 14, 15, 29 and 30 were rejected under 35 U.S.C. §102 (e) as allegedly anticipated by MacInnes et al. (US Patent No. 6,019,984).

Amendments made in this paper and submissions herewith:

In the present Amendment, the specification has been amended to correct minor errors and claims 1, 14, and 29 have been amended to provide clarity. Claims 15 and 30 have been cancelled. Support can be found in the specification for any amendments to existing claims. Applicant's undersigned attorney states that the specification and claim amendments add no new matter.

Rejections under 35 U.S.C. §112, first paragraph:

Claims 1-12, 14-27 and 29-30 stand rejected under 35 U.S.C. §112, first paragraph as allegedly not enabling one skilled in the art to make and use the invention. The claims relate to an antibacterial agent that comprises a nonpathogenic donor bacterial cell harboring at least one transmissible plasmid comprising an origin of replication, an origin of transfer, and optionally, at least one screenable marker gene; wherein the donor cell further comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the plasmid to a recipient cell. The examiner states that the specification does not provide substantive evidence that the claimed antibacterial agent can maintain stability or that the pharmaceutical preparation comprising the antibacterial agent is capable of treating bacterial infections. Applicant respectfully disagrees with the rejection.

Before any analysis of enablement can occur, it is necessary for the examiner to construe the claims. The examiner should always look for enabled, allowable

subject matter and communicate to applicant what that subject matter is at the earliest point possible in the prosecution of the application. (MPEP 2164.04)

The Federal Circuit has consistently held that "the specification must teach those of ordinary skill in the art how to make and use the full scope of **the invention** without undue experimentation. In re Wright, 999 F.2d 1557,1561(Fed. Cir. 1993). Since the invention is obviously that for which patent protection is sought, 'the claims must be analyzed first in order to determine exactly what subject matter they encompass. In re Angstadt, 537 F.2d 498,501 (CCPA 1976). The subject matter there set out must be presumed, in the absence of evidence to the contrary, to be that "which the applicant regards as his invention"' Full effect must be given to all claim limitations. In re Angstadt, 537 F.2d 498,501.

With respect to the present application, the specification teaches antibacterial agents comprising bacterial 'killer plasmids', (e.g. plasmids which undergo runaway replication, or which contain 'killer genes') which are conjugatively transferred from a nonpathogenic donor to a pathogenic recipient. The specification teaches that both *ori* and *tra* sequences are required. The specification further points to several*ori* and *tra* sequences suitable for use in the plasmids of the invention. The specification teaches that conjugation requires contact between the cells and that the transfer of genetic traits can be mediated by many plasmids. Furthermore, the specification teaches that conjugation is among the most efficient natural genetic transfer mechanisms.

Claims 1-12 and 16-27 are directed to antibacterial agents as described above wherein the antibacterial agent comprises a non-pathogenic donor bacterial cell harboring at least one transmissible plasmid comprising an origin of replication for synthesizing the plasmid in a bacterial cell; an origin of transfer from which conjugative transfer of the transmissible plasmid initiates from the donor cell to at least one recipient cell; and optionally a screenable marker.

Claims 1-12 embody a further limitation wherein initiation of replication at the origin of replication is negatively controlled by a plasmid replication repressor; and are further limited wherein the donor cell further comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the transmissible plasmid to the recipient cell, and wherein the donor cell produces the plasmid replication repressor, and further wherein the at least one recipient cell is a pathogenic bacterium that does not produce the plasmid replication repressor, thereby allowing the plasmid to replicate without control ('runaway' replication).

Claims 16-27 are further limited to comprise at least one killer gene that, upon expression in a bacterial cell, produces a product that kills the cell; and are further limited wherein the donor cell further comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the transmissible plasmid to the recipient cell, and wherein the donor cell is modified so as to be unaffected by the product of the killer gene, and further wherein the at least one recipient cell is a pathogenic bacterium that has not been modified so as to be unaffected by the product of the killer gene.

These claims clearly and precisely lay out the limitations of what applicant regards as his invention. None of these claims contain any element or require any limitation relating to 'maintaining stability of the plasmid' or '*in vivo*' use. The applicant respectfully submits that, with respect to these claims, the examiner has either misconstrued the claims or read into the claims a limitation which is not contained within them.

With respect to stability, applicant considers that stability of the plasmid in neither the donor nor the recipient to be critical to the operation of the invention as claimed. In the case of the donor cells, the more important parameter is that the bacteria are capable of conjugatively transferring the plasmid to a recipient cell. Such cells could conceivably be prepared immediately prior to performing the conjugation, wherein there would be substantially little opportunity for plasmid loss from the donor cells. As to the recipient cells, it is expected that the recipient cells would be

substantially prevented from further multiplying, due to the effects of the runaway plasmid or the killer gene transferred to the recipient during the conjugation.

With respect to *in vivo* survival or use, again there is no element or limitation in any of the rejected claims which pertains to *in vivo* use or survival. Accordingly, applicant respectfully requests reconsideration and withdrawal of the Examiner's rejection as to these claims which are fully enabled.

Significantly, the Patent Office Board of Appeals, when presented with similar facts, reversed an Examiner's rejection of claims that related to processes for using monoclonal antibodies to isolate and purify human fibroblasts. In that case, the Examiner's rejection was based on alleged lack of enablement of a screening assay which was not required by the claims. The Board stated:

> The present disclosure as well as that of the parent application does enable one of ordinary skill in the art to practice the ***claimed*** invention. Thus, the claims on appeal are disclosed in the manner provided by 35 U.S.C. 112, first paragraph,. . . and we reverse this rejection of the claims.

Ex parte Erlich, 3 USPQ 2d 1011 (Pat. Off Bd. App. 1987) (emphasis in original).

Moreover, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. (MPEP 2164.04) In this instance, the examiner has stated no reason to doubt the objective truth of the statements contained within the specification and which enable the claimed invention. The Examiner provides the following references which allegedly relate to several factors contributing to the stability of plasmids that are well known in the art.

The Examiner cites Ambozic et al. (Microbiology, 144: 342-353, 1998) as supporting the proposition that a 'natural barrier' effectively bars transfer of a plasmid by conjugal transfer to *Klebsiella pneumoniae*. In those experiments, the researchers were using ColV plasmids. ColV plasmids are large size (145 kb), narrow host range plasmids, known to be harbored only in virulent enteric *E. coli* bacteria. In addition,

they produce both Colla and ColV, toxins both of which are known for killing competing organisms, even closely related ones in the neighborhood of the plasmid bearing bacteria. It is not surprising that such a plasmid would not be efficiently transferred to a non *E. coli* organism even under the selective pressure of two antibiotics. Particularly noteworthy is that the researchers did not first establish whether the 'natural barrier' was either or both of the colicins.

The present invention differs in several significant respects. First, wide host range plasmids are taught, unlike the narrow host range (limited to *E. coli*) ColV plasmid used by Ambrozic et al. Second, any toxic or killing effect of the plasmid would not be present until after the successful conjugative transfer to the recipient. Third, although *Klebsiella* is not specifically claimed, it is contemplated that *Klebsiella* would be an acceptable recipient for a broad host range plasmid. Claims 8 and 23 should be allowable independent of this analysis since*Klebsiella* is not among the limiting group of recipient genera of organisms. Finally, if necessary, it would be trivial to determine whether a particular "killer" plasmid could be transferred efficiently to a particular clinical isolate, as Ambrozic et al. demonstrate in the cited reference.

Next the Examiner cites Roberts et al. (J. Bact., 172: 6204-6216, 1990) to support the notion that specific sequences are required for the complete stabilization of plasmids. Roberts et al. used stabilization sequences derived from a PstI fragment of plasmid RK2, a 60 kb broad-host-range plasmid which is stably maintained in *E. coli* at approximately eight copies per cell. They used plasmid pRR10-ts97, a plasmid which lacked the minimal stabilization region, to assay for stability either short or long-term. They inserted the RK2-derived PstI fragment or portions thereof to determine which sequences were most responsible for optimizing the stability of the plasmid. However, the researchers found the RR10-ts97, which contained none of the inserted stabilization sequences, was only lost at 4 – 12% per generation. While this may not represent a preferred embodiment of the present invention, it certainly establishes that even without these 'complete stabilization' sequences, a loss per

generation of even 10 or 12 % would not render the invention inoperable or unable to be practiced. The observations of Roberts et al. relate to "the broad-host-range character of the RK2 stabilization function and its ability to stabilize efficiently during long-term nonselective growth." These observations in no way preclude or limit the practice of the present invention.

The examiner next cites Klimke et al. (J. Bact. 180: 4036-4043, 1998) to assert that mating stabilization during conjugative transfer between the donor and recipient cells is required. While this study is interesting from a proposed mechanism standpoint, the applicant respectfully submits that it has no bearing on the present application. The claims in question all contain the limitation "wherein the donor cell comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the transmissible plasmid to the recipient cell. . ." The limitation establishes that in order to practice the invention which the applicant is claiming, the required transfer genes must be present. Further, several suitable transfer genes are taught. That this is not difficult in practice to those of skill in the art is self-evident from one of the very problems this invention seeks to solve –the widespread transfer of antibiotic resistant gene from organism to organism across broad species and genus boundaries. Were mating stabilization so difficult to achieve, this problem would not exist. The state of the art of plasmid technology, including conjugative plasmids is high, and it does not require undue experimentation to retain the ability to conjugatively transfer when working with plasmids. There is no suggestion or teaching in the specification that the transfer of plasmid from donor to recipient can occur among nonpiliated Gram-negative cells, nor where the *tra* gene functions are absent.

Finally, the Examiner cites the Rahal et al. (Ann. de Micro. 129:409-414, 1978) to posit that compatibility between the donor cell and the recipient cell is also necessary. Rahal et al. used antibiotic sensitive strains of *Vibrio cholerae* "Eltor" as recipients of twenty-two resistance plasmids from fourteen incompatibility groups and found that only five plasmids were stably inherited in these strains. The other

plasmids were lost at high frequency when the bacteria were grown in nonselective medium. Rahal et al. cannot explain the reason for these results, and speculate that the results reflect why few multiresistant *Vibrio cholerae* have been isolated. Notably they do not rule out the possibility that epidemics of *V. cholerae* carrying R plasmids might be observed in the future.

Again, the studies of Rahal et al. do not preclude or even address the ability of one to make and use the present invention. It is to be expected that some plasmids cannot be conjugatively transferred to certain organisms. It would not require undue or even nonroutine experimentation to determine whether a particular plasmid will transfer from a particular donor to a particular recipient. Moreover, such a transfer need not be 'stable' within the meaning of Rahal et al., as the present invention envisions that the transfer of the plasmid will result in cell death or cessation of normal multiplication. It is not instructive for the purposes of making and using the present invention to consider stable transfer of the plasmids to recipient cells; rather, transfer is to be considered a terminal event in the recipient cell. Neither is it essential to the invention that the plasmid be stable in the donor cell – according to the claims, the donor cell only need possess the ability to transfer the plasmid conjugatively to the recipient. Further, with respect to claims 8 and 23, *Vibrio cholerae* is not a member of the selected group of recipient organisms, and therefore this rejection is not appropriate to those claims which should be allowable independent of this analysis.

As to the Examiner's analysis of the In re Wands factors, the Examiner determined that 1) no declaration under 37 C.F.R. 1.132 or other relevant evidence has been made of record establishing the amount of experimentation necessary; 2) insufficient direction or guidance is presented in the specification with respect to selecting a stable antibacterial agent and pharmaceutical preparation that would achieve a desired level of success when administered to a patient with a bacterial infection that is capable of treating that bacterial infection [sic];. 3) there are limited working examples which suggest the desired results of a [sic] antibacterial agent that is to be used in a pharmaceutical preparation to treat any bacterial infection; and 4) the

relative skill of those in the art is commonly recognized as quite high (post-doctoral level), and the lack of predictability in the field to which the invention pertains is recognized in the art as evidenced by the cited prior art.

Applicant asserts, based on the above detailed response, that no affidavit is required and that undue experimentation is not required. While some experimentation may be useful in specific circumstances, the quantity of experimentation is not dispositive of the analysis (MPEP 2164.04). The key word is "undue," not "experimentation". In re Angstadt, 537 F.2d 498,504 (CCPA 1976). Further, the scope of enablement must only bear a reasonable connection to the scope of the claims. See, e.g., In re Fisher, 427 F.2d 833,839 (CCPA 1970). Applicant further asserts that Examiner's rejection is improperly based on "selecting a stable antibacterial agent and pharmaceutical preparation" when such a limitation is neither part of the claimed invention nor necessary to make and use the invention claimed. As to the limited number of working examples, the specification need not contain a working example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation. In re Borokowski, 422 F.2d 904,908 (CCPA 1970). In the specification at hand, two working examples are provided which each teach how to make and use the invention, including Example 1 which teaches how to make an antibacterial agent of the invention and Example 2 which teaches how to make and use the invention as a pharmaceutical preparation for killing cells. Applicant agrees with Examiner that the typical skill of those in the art is very high, but applicant respectfully disagrees that the art of conjugative plasmids is very unpredictable.

The present invention, as set forth in the claims does not require the complete stabilization of plasmids, as the examiner erroneously contends. While the prior art cited may be useful for inventions requiring the optimization or maximizing of the stability of a plasmid and its transfer, the present invention can be made and used by those of skill in the art without optimizing the stability of the killer plasmid in either donor or recipient.

Where the claimed invention is the application of an unpredictable technology in the early stages of development, an enabling description must provide thoseskilled in the art with a specific and useful teaching. Genentech v. Novo Nordisk, 108 F.3d 1361,1367-1368 (Fed. Cir. 1997). And while tossing out the mere germ of an idea does not constitute enabling disclosure, every aspect of a generic claim certainly need not have been carried out by an inventor, or exemplified in the specification so long as reasonable detail is provided to enable the invention to be understood and carried out. Enzo Biochem v. Calgene, 188 F.3d 1362,1374 (Fed. Cir. 1999).

In the present application, the art of conjugative transfer of plasmids is not an unpredictable technology "in the early stages of development". Rather the insertion of particular killer genes or elimination of a replication repressor are well with the grasp of the skilled artisan in this area.

For the foregoing reasons and explanations, applicant respectfully requests the withdrawal of these rejections under 35 U.S.C. Sec. 112, first paragraph, particularly as they relate to claims 1-12, and 16-27.

With respect to claims 14 and 29 (claims 15 and 30 are cancelled), the examiner's rejection relating to stability does not apply for all the reasons offered above. The specification provides specific teaching related to pharmaceutical preparations on pages 4, and 11-17. The specification teaches which animals the invention is appropriate for, which types of infections by which organisms in which tissues, presently preferred donor cells, presently preferred routes of administration, as well as a list of specific conditions and causative organisms for which the invention is presently preferred. The specification teaches dosage units, and additionally agricultural and horticultural applications as well. With this information in hand, one of skill in the art can make and use the invention either to create an antibacterial agent, or to use such agent in a pharmaceutical preparation for the treatment of a bacterial disease. And while a certain amount of experimentation may be useful, it is not undue as discussed above. The combined teachings of the specification based on the whole of the evidence show that the specification is indeed enabling for the claimed

invention. The specification provides more than adequate guidance to practice the claimed invention. The specification need not be conclusive, but merely convincing to one skilled in the art. (MPEP 2164.05)

Rejections under 35 U.S.C. §112, second paragraph:

Claims 14 and 15 are rejected to as allegedly indefinite for use of the term "pre-determined route of administration". Claims 15 and 30 have been cancelled. The language "pre-determined" in claim 14 (and in claim 29) has been modified in the claims as amended, so as to obviate the grounds of this rejection. The applicant therefore respectfully requests that the rejection be withdrawn.

Rejections under 35 U.S.C. §102:

Claims 1-12 and 16-27 stand rejected as allegedly anticipated by Metcalf et al. (Plasmid 35:1-13, 1996). A claim is anticipated by a reference only if each and every element of the claim is found, either expressly or inherently, in that reference. (MPEP 2131). Moreover, the identical invention must be shown in as complete detail as is contained in the claim. Id.

While Metcalf et al. disclose donor cells which have either pir^+ or pir^-116 genotype, and also teach conjugative transfer to pir^- genotype cells for allele exchange purposes, they do not disclose any plasmids which demonstrate runaway replication in recipient cells which do not produce a plasmid replication repressor, as defined by the claims in the instant invention. Metcalf et al. cannot serve as the basis for an anticipation rejection for the claims as amended.

The disclosure of Metcalf et al. does not address or consider a donor cell which produces a plasmid replication repressor nor a recipient cell which does not possess the plasmid replication repressor - to create the runaway replication plasmid

of claim 1, and claims which depend from it. Furthermore, Metcalf et al. do not teach a plasmid with at least one killer gene which upon expression produces a product that kills the cell, nor a donor cell modified so as to be unaffected by the product of the killer gene, nor a recipient cell which is a pathogenic bacterium not modified so as to be unaffected by the product of the killer gene – to create the killer plasmid of claim 16 and claims which depend from it.

Claims 14-15 stand rejected as allegedly anticipated by MacInnes et al. (US Patent No. 6,019,984). As stated above, a claim is anticipated by a reference only if each and every element of the claim is disclosed in the reference. (MPEP 2131).

MacInnes et al. disclose microbial preparations for the production of vaccines comprising microbial cells. The microbial preparations may be prepared by the use of genetic techniques, including conjugative plasmid transfer, but the plasmids taught by MacInnes et al. do not contain a killer gene, which limitation is present in claim 16 and claims that depend from it, including amended claim 29. In addition, MacInnes et al. do not disclose plasmids as in the amended claims, wherein the plasmid causes runaway replication in the recipient cells. The teachings of MacInnes et al., therefore cannot anticipate the claims of the present invention.

In light of the foregoing arguments and for all the reasons laid out above, the applicant respectfully asserts that all claims as amended are novel over the cited references. Accordingly, withdrawal of the rejections under 35 U.S.C. Sec. 102(b) and 102(e) based on Metcalf et al. or MacInnes et al., respectively, is requested.

Summary

In view of the foregoing amendments and remarks, the applicant submits that this application is in condition for allowance and respectfully request early and favorable notification to that effect. If it would expedite prosecution of this application, the Examiner is invited to confer with applicant's undersigned representative.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 12, line 18, has been amended as follows:

In addition, other types of killer genes may be utilized similarly. These include naturally-occurring or synthetic genes. A nonlimiting example of a naturally-occurring gene that is suitable for use in the invention is the *hok* gene product described by Gerdes et al. (Gerdes, K., Bech, F., Jorgensen S., Loebner-Olsen, A., Rasmussen, P.,Atlung, T., Boe, L., Karlstrom, O., Molin S., and von Meyenburg K. 1986. Mechanism of postsegregational killing by the *hok* gene product of the *par*B system of plasmid R1 and its homology with *rel* F gene product of the E. coli *rel*B operon. EMBO J. 5: 2023-2029). Examples of man-made nucleic acid molecules that may be used in this aspect of the invention include: (1) sequences encoding non-hemolytic β-amino acid oligomers, which are a new class of <u>molecules</u> based on inhibitors of Sigma-Core RNA polymerase interaction; (2) sequences encoding peptides with bactericidal activity and endotoxin neutralizing activity for Gram-negative bacteria as described in U.S. Patent 5,830,860; (3) sequences encoding RNA molecules with binding affinity to critical bacterial cellular targets (e.g., Chen, H., Gold, L. 1994. Selection of high affinity RNA ligands to reverse transcriptase: Inhibition of cDNA synthesis and Rnase H activity. Biochemistry 33: 8746-8756); and (4) oligonucleotides generated by the *SELEX* method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules as described in U.S. Pat. No. 5,475,096 and U.S. Pat. No. 5,270,163.

IN THE CLAIMS:

1. (Amended) An antibacterial agent, which comprises a non-pathogenic donor bacterial cell harboring at least one transmissible plasmid comprising:

a) an origin of replication for synthesizing the plasmid in a bacterial cell, wherein initiation of replication at the origin is negatively controlled by a plasmid replication repressor, wherein in the absence of the plasmid replication repressor the transmissible plasmid undergoes runaway replication;

b) an origin of transfer from which conjugative transfer of the transmissible plasmid initiates from the donor cell to at least one recipient cell; and, optionally,

c) at least one screenable marker gene;

wherein the donor cell further comprises one or more transfer genes conferring upon the donor cell the ability to conjugatively transfer the transmissible plasmid to the recipient cell, and wherein the donor cell produces the plasmid replication repressor, and further wherein the at least one recipient cell is a pathogenic bacterium that does not produce the plasmid replication repressor, thereby enabling the transmissible plasmid to undergo runaway replication in the recipient cell.

14. (Amended) A pharmaceutical preparation for treating a patient for a bacterial infection, comprising the antibacterial agent of claim 1 formulated for a ~~pre-determined~~ route of administration to the patient, wherein the route of administration is selected from the group consisting of: topical, oral, nasal, pulmonary, ophthalmic, aural, rectal, urogenital, subcutaneous, intraperitoneal and intravenous.

29. (Amended) A pharmaceutical preparation for treating a patient for a bacterial infection, comprising the antibacterial agent of claim 1 formulated for a ~~pre-determined~~ route of administration to the patient, wherein the route of administration is selected from the group consisting of: topical, oral, nasal, pulmonary, ophthalmic, aural, rectal, urogenital, subcutaneous, intraperitoneal and intravenous.